White Paper

Lipogro® Ultra-Low IgG Cholesterol Concentrate

Maximize your research investment.

Lipogro® Cholesterol Concentrate solution is a cell culture media supplement used for the growth of cell lines or as an additive in monoclonal antibody production. Lipogro® may be used as a Fetal Bovine Serum (FBS) substitute, in combination with FBS or Newborn Calf Serum (NBCS) to reduce the amount of serum needed, or as a cell culture supplement for serum-reduced or serum-free media.

Lipogro®, available in multiple sizes, is available in Australia & NZ origin or domestic (USA) origin.

Lipogro® is manufactured in a state-of-the-art facility adhering to GMP standards, stringent manufacturing controls and validated CIP cleaning processes. Derived from the lipid fraction of an adult bovine serum, Lipogro® is a high-clarity aqueous solution rich in lipoproteins (including purified cholesterol and essential fatty acids)

which increases cell growth, viability and productivity. Lipogro® has been used as a cell culture supplement for Myeloma (NSO, Sp20), Hybridoma, HEK293, MDCK, Vero, MRC-5 and CHO cell lines, in the production of veterinary vaccines, monoclonal antibodies, recombinant proteins and live cellular therapies

RMBIO manufactures Lipogro® using a proprietary process and strict quality control guidelines. The process uses a series of extraction and filtration steps (to retain maximum product potency), two heating steps (for viral inactivation) and 0.1 µm filtration (for sterility).

Lipogro® is tested for sterility, the presence of viral and bacterial contaminants as well as physical and chemical characteristics

Lipogro® Specifications

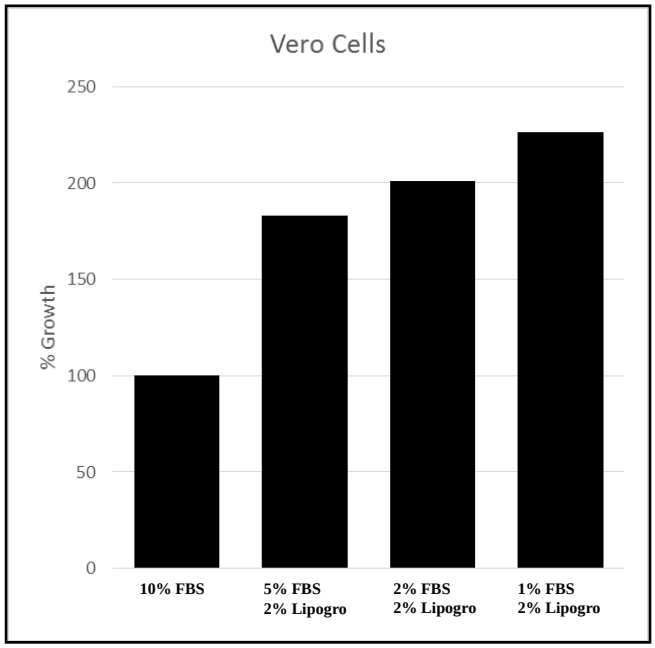
Source	US, Australian or NZ Adult Bovine Serum
Appearance	Clear Amber Solution
Total Cholesterol	9-11 g/dL
Total Protein	< 2 g/dL
Endotoxin	< 5 EU/ml
Mycoplasma	None detected
Sterility (Bacteria & Fungi)	No Growth
Virus (9 CFR 113.53)	
Bluetongue	None Detected
Bovine Adenovirus	None Detected
Bovine Parvovirus	None Detected
Bovine Respiratory	None Detected
Bovine Viral Diarrhea Virus	None Detected
Rabies	None Detected
Reovirus	None Detected
Cytopathogenic (e.g. IBR)	None Detected
Hemadsorbing (e.g. PI3)	None Detected
pH (7% Solution)	7.0-8.4
IgG	None Detected
Storage Temperature	2-8°C
Shelf Life	24 months

Lipogro® is available in Grade A (serum sourced from Australia and New Zealand) and Grade B (serum sourced from the United States), and has been shown to be safe for use in pharmaceutical applications. RMBIO produces Lipogro® in large batch sizes, yielding superior lot-to-lot consistency and assuring a high-performing product.

Cell adaptation to reduced FBS with Lipogro®

Vero cells were quick-adapted to reduced FBS concentration with Lipogro® supplementation. In this process, a confluent plate of cells was split 1:4 and the cells allowed to adhere for 16-24 hours in 10% FBS EMEM. The media containing 10% FBS was removed, replaced with media containing 0.5% FBS and the cells grown for an additional 16-24 hours. The low serum media was removed and replaced with base media containing 5%, 2% and 1% FBS supplemented with 2% Lipogro® (by volume). Initially, cells were sub-cultured at 1:2 every 2-3 days for several weeks, and then sub-cultured at 1:8 once per week.

Cells were plated at 2500 cells/well (96-well Tissue Culture treated plate) in their respective media (10% FBS or 5%/2%/1% FBS + 2% Lipogro®), MTS reagent added after 1 or 70 hours and absorbance quantified. The difference in absorbance between 0 and 72 hours was compared and relative growth assessed.

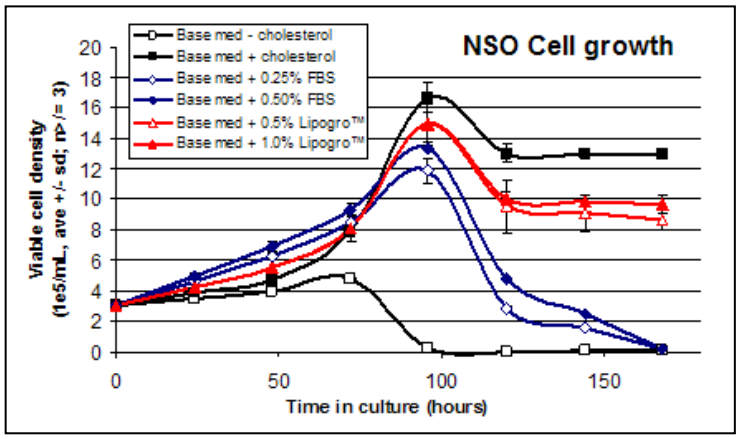


Lipogro® increases cell response under reduced-serum conditions

Vero cells grown under reduced serum concentrations in the presence of 2% Lipogro® showed greater growth than cells grown in 10% FBS alone.

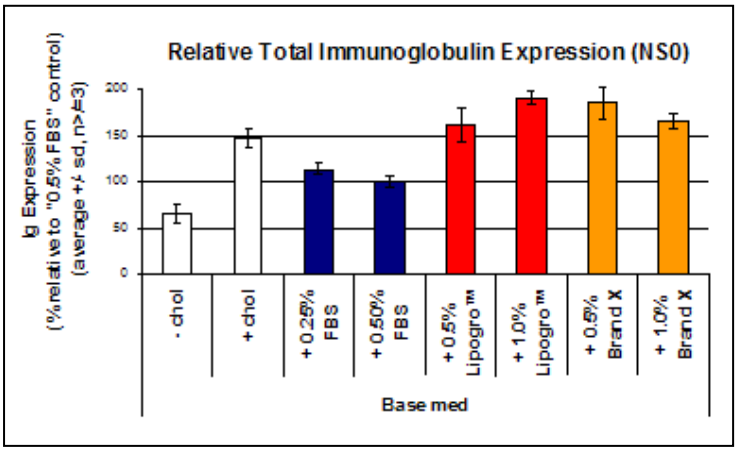
Growth and IgG production by NS0 cells

NS0 cells were grown in base medium supplemented with a standard cholesterol, FBS or Lipogro®, and the density of viable cells assessed over time. Supplementation of the medium with standard cholesterol resulted in a 16-fold increase and 0.5% & 1% Lipogro® a 15-fold increase in viable cell density at 100 hours (cells grown in the absence of cholesterol were not viable).



Lipogro® increased the number of viable NS0 cells over control medium and medium supplemented with FBS alone

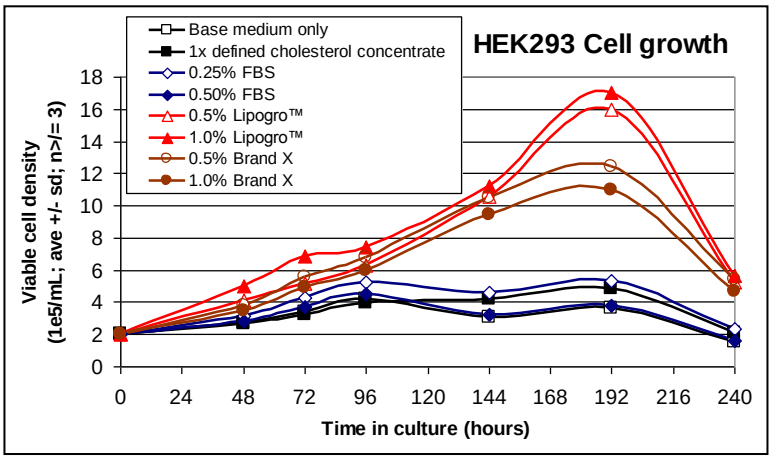
Secretion of IgG was compared for NS0 cells grown in the presence/absence of standard cholesterol, FBS and Lipogro. NS0 cells grown in media supplemented with a standard cholesterol (brand X) showed a ~115% increase in IgG, while cells grown in the presence of Lipogro® showed a ~140% (0.5% Lipogro®) and 170% (1% Lipogro®) increase in IgG production over the no cholesterol control.



Lipogro® enhanced expression of IgG by NS0 cells over medium supplemented with standard cholesterol or with FBS

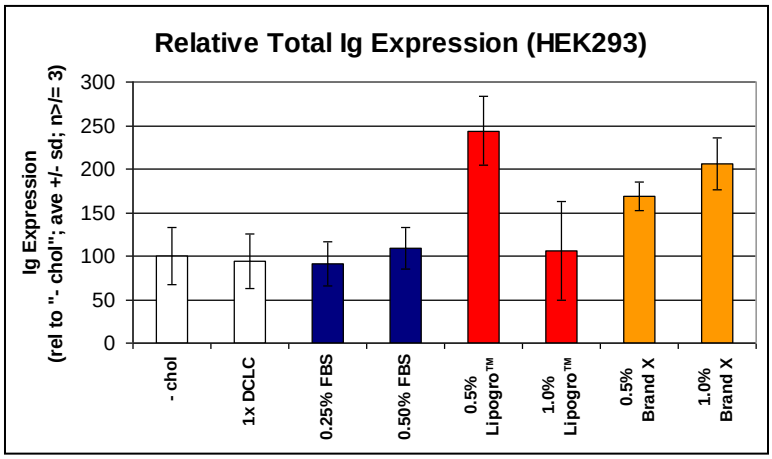
Growth and IgG production by HEK293 cells

HEK293 cells were also grown in base medium supplemented with standard cholesterol, FBS or Lipogro®, and the density of viable cells assessed over time. Supplementation of the medium with standard cholesterol resulted in a 5-fold increase and 0.5% & 1% Lipogro® a 16- & 17-fold increase in viable cell density at 192 hours.



Lipogro® increased the growth of HEK293 cells over control medium and medium supplemented with cholesterol or FBS

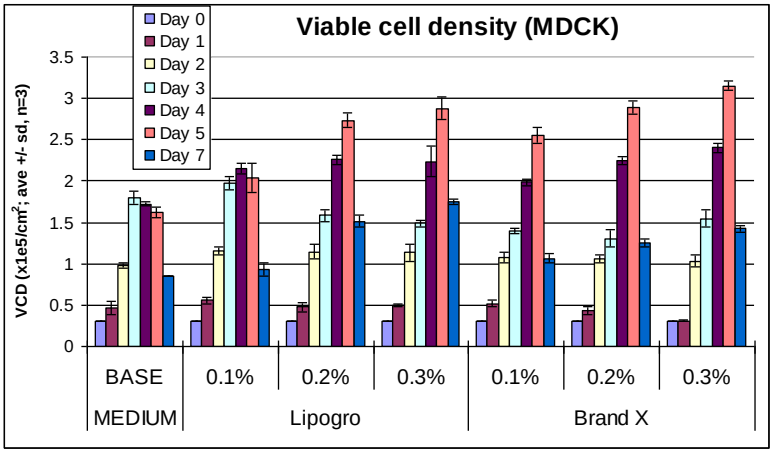
Secretion of IgG was compared for HEK293 cells grown in the presence/absence of cholesterol, FBS and Lipogro. Cells grown in media supplemented with Lipogro® showed a ~150% (0.5% Lipogro®) increase in IgG production over the non-cholesterol containing control.



Lipogro® enhanced expression of IgG by HEK293 cells

Growth of MDCK cells

MDCK cells were grown in base medium supplemented with Lipogro®, and the density of viable cells assessed over time. Supplementation of the medium with cholesterol resulted in a ~2-fold increase in viable cell density at 5 days.



Lipogro® increased the growth of MDCK cells over base medium

Summary

Medium supplemented with standard cholesterol or with Lipogro® showed increased viable cell densities in all the cell lines tested, compared against medium supplemented with FBS alone. Cells grown in media supplemented with Lipogro® showed higher relative IgG secretions than cells grown in media supplemented with standard cholesterol or FBS alone.